Synergism Effect of Antibacterial Activity of Some Medicinal Plants

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Abstract
Synergism between plant extract was carried out in present study using sex common plants which extracted by mixture of methanol: water (20:80)%, then it characterized by thin layer chromatography, antimicrobial activity of these extract performed using OD of bacterial growth at 600 nm against Escherichia coli, Staphylococcus aureus, Serratia spp., Klebsiella pneumoniae, Aeromonas hydrophilla isolation from different source in hospital tests were performed as only one extract on every bacterial species then tow extract was mixed together for evaluated synergist efficiency on every bacterial species, results show that used one extract only causes decreased in bacterial growth in different level, barley was the more effect on pathogenic bacteria but sage was lower effect, it caused activated bacterial growth. Synergism between plant extract also show different effects level according to mixture forming and types of bacterial species. Present study concluded that mixture of plants used in herbal medicine have disadvantages in addition of its advantage to treatment bacterial infection, in other hand synergism between plants may be more effects.

Keywords: synergism, OD, pathogenic bacteria.

Introduction
Return to the nature is becoming important idea in the last decades, because of increasing side effects of drugs, drug tolerance in patinas and new recompenants in genetic materials of bacteria which is responsible of drug resistance. The variation in phytochemicals compounds in different species of plants give us ability to use this materials in different application, like industrial, economic and medical application (Al-Saadi, 2012). The medical applications of plant are important but it must be under the physicians recommendations forlowering level of its side effects.

Review of literature clarify the plants extracts roles as anti-bacterial agents in different site and pathogenesis. Mahesh and Satish (2008) used methanol leaf extracts of Acacia nilotica, Sidacordifolia, Tinosporacordifolia, Withania somniferand Ziziphus mauritiana showed significant antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis pv. malvacearum by inhibition zone method. Also some Indian traditional plant extracts such as Ocimum sanctum, Ocimum gratissimum, Aeglemarmelos, and Adhatodavasica leaves showed antibacterial activity against human pathogenic Bacteria (Prasannabalaji et al., 2012).

Researches uses different method to extract phytochemicals compounds such as watery (cold and hot), organic solvents, directed and fractionation methods in order to obtain maximum concentrations of compounds. Plants that used in present study was used in wide rang in medical applications, such as anti-bacterial, anti-fungus, antioxidant and anti-mutagenesis. Also studies show that side effects of this plants extracts was low than industrial drugs.

1- Licorice (glycyrrhizaglabra)
Licorice (L) was one of the important plants have long history in medical applications, root is important part in plant which is contained flavonoid components (Yamamura et al., 1992). Licorice also contain phenolic compounds, coumarins, volatile oil, saponine and minerals (AL-Turiahe, 2010). Fukai et al., (2003) improve that licorice useful in treatment bacterial infections of Streptococcus mutants, Staphylococcus aurus and Mycobacterium. Also it have antibacterial activity against G+ and G- bacteria such as Bacillus subtilis, MTCC121, Salmonella typhi MTCC733, Yersinia enterocolitica and Klebsiella pneumoniae MTCC (Khamja, et al., 2006).

2- Barley (Hordeum vulgare L.)
Barley (B) is one of the importance food sources in the world, all part of plants fruit, leaves, seeds, grains and whole plants has been used in different medical treatments (Marwat et al., 2012). Barley contain from soluble and insoluble dietary fiber (DF), vitamins E and C, B-complex vitamins, minerals and phenolic compounds, highly visoce of soluble polysaccharide (Mahesh et al., 2010). Many studies supported using barley in different inflammation such as Intranasal inflammation by use in Iran (Ross, 2005).
3- ORIGANUM VULGARE
   *Origanum vulgare* (O) used as antioxidant and anti-mutagenesis activity in many researches also in many
   studies it used many compounds that extracted by organic solvent such as anethol, methanol and water
   from different parts of plants. Ashraf *et al.* (2011) used equas and chloroform to extract
   phytochemicals compound from *Origanum vulgare* and used it as antibacterial and antifungal agents.

4- Salvia officinalis L
   *Salvia officinalis* L (S) or it is known sage, genus from Lamiaceae family, using as flavoring agent in
   food preparation, and as medicinal plants using in different applications (Stanojevic *et al.*, 2010). Khalil
   and Li (2011) extract essential oil from *Salvia officinalis* and used it as antimicrobial agent, in the other
   hand many studies use extract of sage as antibacterial agents against gram+ and gram- bacteria
   (Behboud *et al.*, 2011). Stefanovic *et al.* (2012) use synergism between sage and *CICHORIUM
   INTYBUS* against pathogenic bacteria.

5- Zingiber officinale
   Commonly known as ginger (G), rhizome was the important part of plant that use in medical
   application, studies clarified that extract of ginger consist from phenols, volatile oils (Nanjundaiah
   found that thanthmanol extract was more efficient than other extract in disc diffusion methods.

6- Pomegranate
   *Punica granatum* (P) its known fruit that used in different food applications, the cortex offruit contain
tannins such as punicalin, punicalagin and granatin B also it contain alkaloids like pelletierine , pseudo
pelletirine and methyl pelletirine (Al-saadi *et al.*, 2012) literature of review reported the Pomegranate
   efficiency against pathogenic bacteria *B. coagulans*, *B. cereus* *B. subtilis* and *S. aureus* (Dahham *et al*.
   2010).

   The aim of this study is evaluation synergism between plant extract against Pathogenic bacteria
   *E.coli*, *S. typhi*, *s.aureus*, *Serratia*, *Klebsillapnemionae*, *Aeromonas hydrophilw* was isolated from different
   infection.

**Materials and methods**

1- Plant part that use in present study, licorice deried root, barley seeds, ginger dreid rhizomes, punica
cortex, leaf of *ORIGANUM VULGARE* and sage.

2- **Plants extract**: The plants extracts of plant was prepared according to the method of Sato *et al.* (1990)
   with some modification. Specific weight of the plant and it is mixed with the average 1 gm to 3 ml of
   the solvent solution (20 %methanol: 80 % distilled water), The mixture is uniformed by electric
   blender for 30 minutes in room temperature. The solution is filtered by using gauze fabric for getting
   transudate solution. It is deried using incubator at 50C° for 24 hours, Thedispert is kept in a dry place
   until it is used.

3- **Plant extract aliquot**, it prepares in 3 concentration (0.1 g/ml) then it sterilized using mellipor 0.2
   mm.

4- **TLC profile**; plant extract characterized by TLC, by use solvents (DW: ethylacetate: mthanol)
   (20:60:20 v/v/v) as mobile phase, then bands exam in visible and UV light in 312 wave length to detect
   retardation factor.

5- **Pathogenic bacteria** *E.coli*, *S.aureus*, *Serratia Spp.*, *Klebsillapnemionae*, *Aeromonas hydrophilw*
   isolated from different source in hospital in suitable media, then antibiotic sensitivity was detected
   using disc methods to (*mecillin ME*, amikacine AM, azithromy cine AZM, fusicid acid Fa,
imipenem IPM, nalidixic acid NA, piperacillin PRL, ampicilline/ceoxacillin APX, doxycycline DO,
nitrofurantion F, oxacillin OX, rifampin RA, clindamycin PA and trimethoprim/sulphamethoxazol
   SXT).

6- **Antibacterial activity of plant extract**; this assay performed using OD of grothculture according to
   Jabor *et al.* (2013).

7- **Synergism of plant extract**; this assay performed using mixed between tow plant extract as, Every
   mixed in this mixture was experiment against pathogenic bacteria in 5 microgram/ml media for every
   type of plant extract.

**Results**

Thin layer chromatography of plants extract show these extracts consist of different compounds as show in
figure (1) under visible light and UV light.

The method that used in present study for extract plants is common and used in wide range of researches because methanol and water was high polar solvents may extract polar phytochemicals compounds, also this mixture of
solvent was low toxicity if it used in vivo (Al-turaihe et al. 2012, AL-saadi et al. 2013).

Figure (1) thin layer chromatography of plant extract using as antibacterial activity under visible and UV light 
A, barley ; B, Liquors; C, Origanum vulgare ; D, Pomegranate cortex; E, Ginger; F, sage.

Antibacterial activity

Plant extract used as anti-bacterial activity against five type of pathogenic bacteria, include E.coli; S.aureus; Klebsillapnemoniae ; Serratia Spp; Aeromonashydrophilliwas, its sensitivity against antibiotics was performed using disc method as show in table (1).

Results show that barley had antibacterial activity against all pathogenic bacteria that used in present study, as show in column 3 in all figures, this may be becaus e it have different compounds in it extract that effect on bacterial growth, studying clarify that barley consist of soluble and in soluble fiber and bioactive constitutes such as vitamins and minerals, cellulose beta-glucan, so it a source of tocols compounds (Marwat et al., 2012).

Barley has historical uses in medical applications partially in inflammation and infection in different country such as renal inflammation, respiratory tract infections and intranasal inflammation. (Ross, 2005; Bussmann et al., 2007). The lower effect from these plant extract was Salvia officinalis Lit increased growth of bacteria except S.aureus which sensitive to this plant , this may be because these plant have supported compounds for bacterial growth these result in fall in to tow suggestion first , sage can enhancement infection of pathogenic bacteria thus it must be avoided in infection disease, in another hand it need to others studies using different solvent in extract
and nonpathogenic bacteria. Khalil and Li (2011) used Salvia oil as antibacterial against some gram positive and gram negative bacteria, they found that the essential oil of S. officinalis proved to have antibacterial activity against Gram- positive and negative bacteria. This activity was more obvious against Gram-positive than negative bacteria which deal with present study. This may be due the existence of the outer phospholipid membrane of the gram-negative bacteria.

Other plant extract have low activity for all species of bacteria such as licorice (lane 2) it cause low effect on bacterial growth. Hordeum vulgare more effect on staph. And Aeromonashydrophillia than others types, Punica granatumum effect on Aeromonashydrophillia and staph aureus, ginger effect on three species, Aeromonashydrophillia, serrata and staph, this result deal with many studies in other countries Dahham et al (2010) used different extract of Punica granatumum against human pathogenic bacteria they concluded, that results obtained from their study clearly demonstrate broad spectrum antimicrobial activity of pomegranate against seven species of bacteria, More importantly the results indicated that methanolic extracts of pomegranate are more effective against bacteria and fungi than the aqueous extracts so this effect may be because presence of phytochemicals compounds in thee extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities.

Sebiomo et al (2011) used serial concentrations of different types of ginger extract against Staphylococcus aureus and Streptococcus pyogenes results show that ethanol extract of both leaf and root can be used alongside conventional antibiotics to fight agents of infections that are so prevalent in the hospitals.

Present study proposed synergism between plant extract against pathogenic bacteria, this suggestion mimic mixtures of herbal medicine have been used in treated disease, and results of present study clarify effect of this synergism against pathogenic bacteria, figure (3) show synergism between plant extract against pathogenic bacteria, in all diagrams in figure (3) lane (1) is positive control, lane (2) is mixture of barley and ginger more effect was against Aeromonashydrophillia and S aureus. Lane (3) is barley and Pomegranate the more effect was against Aeromonashydrophillia and kliepsella also it has effects on all bacteria it decreased growth, lane (4) is ginger and Origanumvulgare more effect of this mixture on Aeromonashydrophillia and no effect on Klebsillapnemoniae it activated its growth, so it has low effect against other bacteria, lane (5) is Pomegranate and ginger this mixture causes decreased in all bacterial growth and complete inhibition on Aeromonashydrophillia, lane (6) Origanumvulgare and barley it causes low effect on all bacterial species, lane (7) sage and barley this mixture also decreased all bacterial growth but more effect was o AREOMOAS , lane (8) is barley and liquorice, also Aeromonashydrophillia was more affected by this mixture, lane (9) is liquors and Origanumvulgare the result of this mixture was like others in more effect on Aeromonashydrophillia and low effect on others, lane (10) is liquorice and Pomegranate causes inhibition in Aeromonashydrophillia and decreased in other bacterial species, lane (11) is Origanumvulgare and sage also it cause decreased in Aeromonashydrophillia and S aureos growth and E coli, lane (12) is liquorice and sage its effect on E.coli and Aeromonashydrophillia so it effect on others bacteria but in low effect, lane (13) Pomegranate and liquorice it decreased bacterial growth but inn low level, lane (14) liquorice and ginger causes complete inhibition on Aeromonashydrophillia and most decreased in bacterial growth especially in E.coli, lane (15) Pomegranate and sage it caused decreased in all bacterial growth, lane (16) sage and ginger it also decreased in bacterial growth. These results were resulted from synergism between plants extract mixture which contain phytochemicals compounds may be active if combine with other compounds or these compounds have sequential effect on its target in bacteria, also these compound may be changes microenvironmental of bacteria which causes disrupted in nutrient and water transfer, this reasons need to advance studying using electronmicroscope to understand these effect on bacterial cell wall and in microenvironment.

Some study use synergism between plant extracts and antibiotics, Adwan and Mehanna (2008) use different plant extract with antibacterial had different mechanism such as protein synthesis inhibition: oxytetracyclineHCl and gentamycin sulfate; cellwall synthesis inhibition: penicillin G and cephalaxin; folic acid synthesis inhibition: Sulfadimethoxine assodium; and nucleic acid synthesis inhibition: enrofloxacinagainst Staphylococcus aurous Strains Isolated from Clinical Specimens results of this studying were that synergistic effects between combination of antibiotics and plant extracts with significant reduction inthe MCIs of the test antibiotics against these strains of S. aurous, these results deal with present study results in synergist plant extract with other compounds if it was antibiotics or other extracts.

Present study suggested use synergism between plant extract as antibiotics or used it for make new drug sesspecially against multi resistant bacteria, which become dispersed in hospital, environment causes different infections and disease.

Synergism between plant extracts in present study also have disadvantage, sometimes it causes activating bacterial growth ashow in figure 3 lane (8) against E. coli and lane (4) against Klebsillapnemoniae and lane(2) in serrata while when it use alone it causes inhibition in bacterial growth, such as barley it cause inhibition in E. coli, Origanumvulgare when it use alone also it causes activate bacterial growth of Klebsillapnemoniae, so ginger has low effect on this bacteria.
Synergism between plant extract as treatment or protect factors were evaluated in variety studies many of this studies recorded plant extract synergism efficiency in its effected, but these studies on pathogenic bacteria has been little and need to advance techniques in extracted phytochemicals compounds and experience these extract on bacterial genome and plasmid curing.

Figure (2) antibacterial activity of plants extract against different pathogenic bacteria
A. E.coli; B. S.aureus; C. Klebsiilla pneumoniae; D. SerratiaSpp; E. Aeromonashydrophillias.
1. positive control; 2. glycyrrhiza glabra; 3. Hordeum vulgare; 4. Origanumvulgare; 5. Salvia officinalis;
Figure (2) antibacterial activity of plants extract against different pathogenic bacteria
A. *E.coli*; B. *S.aureus*; C. *Klebsiella pneumoniae*; D. *Serratia Spp*; E. *Aeromonas hydrophilia* was.


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